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TITLE: Lowering T Cell Activation Thresholds and Deregulating Homeostasis to Facilitate Immunotherapeutic Responses to treat Prostate Cancer

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## ABSTRACT.

The induction of tumor-specific T cells remains a primary obstacle to immunotherapeutic approaches for most cancers including prostate cancer. This difficulty has been largely ascribed to mechanisms for tumor evasion of the immune system and host-imposed restrictions (collectively referred to as tolerance) that prevent cross-reactive autoimmunity against the parent tissues from which tumors arise. Limitations in techniques to identify novel and truly immunogenic prostate-specific antigens and efficient methods to modify autologous tissues for vaccine preparation have further constrained approaches to develop immune-based therapies for prostate cancer. Hence, relatively straightforward manipulations that induce specific T cell responses against prostate tumors or epithelial tissues, especially in vivo, might ultimately prove valuable for prostate cancer immunotherapy. Our studies explore a new paradigm in which we will exploit blockade of T cell purigenic receptors A2a and A2b (using caffeine) to alleviate tumor-induced impairments in T cell function to potentiate T cell-mediated immunotherapeutic responses to treat established prostate tumors in mice.

## INTRODUCTION.

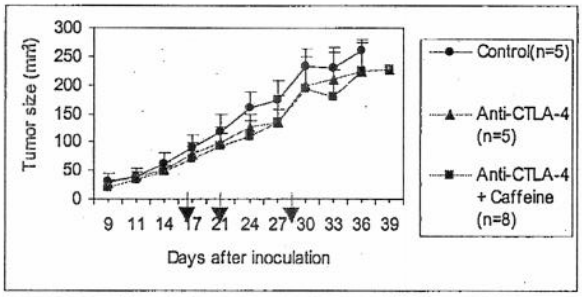
In our original application, we proposed three Aims. In Aim 1 we originally proposed to elucidate mechanism(s) whereby AA lowers costimulatory T cell activation thresholds. In Aim 2 we proposed to determine whether AA lowers T cell activation thresholds in men with prostate cancer. In our original Aim 3 we proposed to test whether combination regimens incorporating AA plus chemotherapy can facilitate synergistic responses to immunotherapy to improve prostate cancer treatment.

In April 2004, we requested and were granted, permission to modify Aims 2 and 3 of our original proposal to eliminate scientific overlap with an RO1 award that we received in 2004. Hence, in a revised SOW, we proposed to exploit caffeine-mediated blockade of T cell purigenic receptor in combination with CTLA-4 blockade in order to enhance T cell responses to treat advanced prostate tumors in mice. The rationale for these experiments emanated from recent studies reported by Sitkovsky et al suggesting that full-blown anti-tumoral T cell responses may be severely inhibited by high intratumoral concentrations of adenosine, and that blockade of T cell purigenic receptors A2a and A2b may alleviate tumor-induced impairments of T cell function.[1,2] Specifically, it had long been recognized that "established" tumors produced relatively high concentrations of intratumoral adenosine that emanate from shifts in oxidative to anaerobic glycolytic respiration within the hypoxic intratumoral microenvironment. Consequently, large and relatively hypoxic tumors were anticipated to produce higher levels of adenosine. Dr. Sitkovsky's group had also published that naïve T cells constituitively expressed A2a adenosine receptor and activated T cells further expressed both A2a as well as A2b adenosine receptors. Blockade of T cell adenosine receptors by a relatively non-specific purigenic antagonist, caffeine, was shown to markedly potentiate T cell responses both in vitro and in vivo. Finally, it has been demonstrated that T cells from A2 receptor-deficient transgenic mice could mediate complete rejection of established tumors following their adoptive transferred into the tumor-bearing host. Collectively, these findings strongly suggested that adenosine produced by established tumors might inhibit T cell responses by binding to T cell purigenic receptors which, in turn, downregulate T cell-mediated antitumoral immunity.

Prompted by these observations, we proposed to investigate whether T cell purigenic receptor blockade might enhance the effectiveness of various forms of immunotherapy to treat very established tumors in our prostate cancer murine models. Specifically, in our new Aim 2, we proposed to elucidate mechanism(s) whereby adenosine inhibits costimulatory T cell activation and the mechanism(s) whereby caffeine-mediated purigenic receptor blockade promotes costimulatory T cell activation. In our new Aim 3 we proposed to test whether in vivo caffeine-mediated blockade of T cell purigenic receptor plus immunotherapy can act synergistically to improve prostate cancer treatment.

**BODY.**

We previously completed the objectives outlined in our original Aim 1 and revised Aim 2. By mid 2005, we had identified the optimal (highest) dose and formulation to test whether caffeine-mediated T cell purigenic receptor blockade could facilitate immunotherapeutically-induced T cell-mediated responses directed against established ectopic and autochthonous prostate tumors in the transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model. Our experiments demonstrated that mice receiving 5 mg subcutaneous time-release tablets tolerated treatment well. Hence we initiated studies outlined in Aim 3 to test whether in vivo caffeine-mediated blockade of T cell purigenic receptor plus immunotherapy might act synergistically to improve prostate cancer treatment or antitumoral therapy in general.

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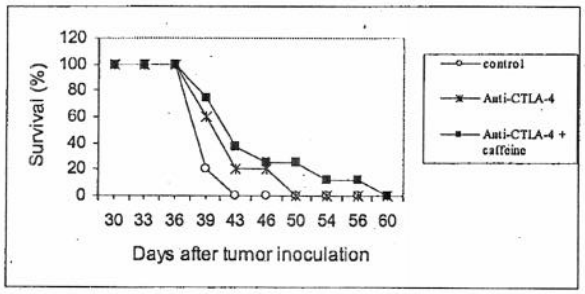


**B**

**Figure 1** In our first series of experiments, we endeavored to treat mice with established subcutaneous Tramp C1 tumors (murine prostate carcinoma) with systemic purigenic blockade (provided by 5 mg sub Q caffeine pellets) combined with CTLA-4 blockade; a form of immunotherapy that we have previously examined. Treatment of these mice was not initiated until tumors achieved a mean dimension of approximately 75 $mm^2$ (typically around day 17 following tumor cell innoculation). Additional controls for these experiments included treatment with irrelevant IgG as well as "placebo" subcutaneous vehicle pellets (controls not shown). All cohorts were comprised of 5 mice and experiments were repeated x 3. As is shown in **Figure 1**, we consistently observed no advantages to purigenic blockade of mice using caffeine as a means to facilitate Tramp C1 tumor regression in response to CTLA-4 blockage immunotherapy.

Given that these experiments were being conducted using mice bearing highly established tumors (as opposed to early tumors that are still developing after tumor cell innoculation), we postulated that perhaps a more rigorous form of immunotherapy might be required in order to facilitate the regression of highly established TC1 tumors. Thus, experiments were repeated using systemic purigenic receptor blockade + CTLA-4 blockade + GM-CSF TC1 vaccination. The basis for this approach was predicated on our prior demonstration that the combined CTLA-4 blockade + GM-CSF TC1 vaccination was sufficient to induce autoimmune prostatits in normal C57/BL/6 mice.[3] Moreover, this combination of therapy has previously been reported to induce vitiligo like hair depigmentation in C57BL/6 mice being treated for B16 melanoma.[4] Thus experiments were

performed as above with the addition of GM-CSF TC1 vaccination to assist with priming of T cell responses against TC1 tumors. Treatments were repeated every 3 days x 4. Additional controls including irradiated TC1 vaccine, irrelevant IgG & placebo pellet placement were also included in all experiments (not shown). All experiments were performed x 3. All cohorts were comprised of 5 mice.

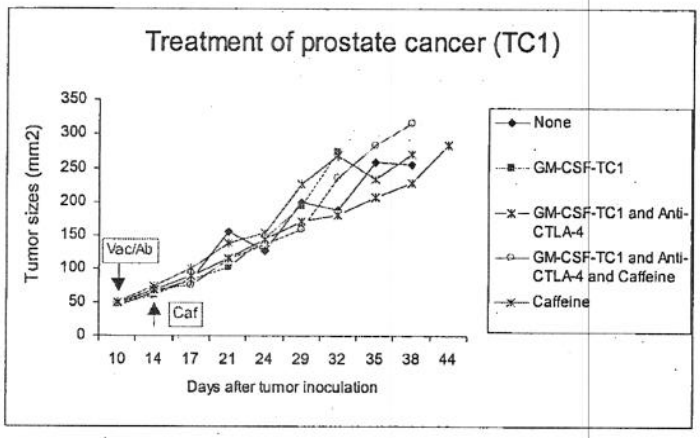


**Figure 2**

As is evident in **Figure 2**, the addition of systemic purigenic blockade failed to enhance the effectiveness of GM-CSF tumor cell vaccination alone, or when given in combination with CTLA-4 blockade. In fact, none of the immunotherapeutic treatments tested proved effective against relatively established TC1 tumors.

In light of this, we then chose to explore the impact of systemic purigenic receptor blockade in the context of promoting antigen-specific T cell responses against tumors with defined nominal "tumor antigen". Specifically, we tested whether systemic purigenic receptor blockade (in the form of caffeine pellet treatment) could augment responses against OVA-expressing B16 tumors (EG7) in normal C57BL/6 mice adoptively receiving 3 x $10^6$ OT-I CD8+ T cells specific against OVA antigen. Additionally, some mice received CTLA-4 blockade x 4 (every third day) following OT-I T cell transfer given on Day 15 when tumors were roughly 150 $mm^2$. Again, additional control groups were included for these experiments including adoptive transfer with equivalent amounts of purified normal CD8+ T cells, placebo pellets and irrelevant IgG (not shown).

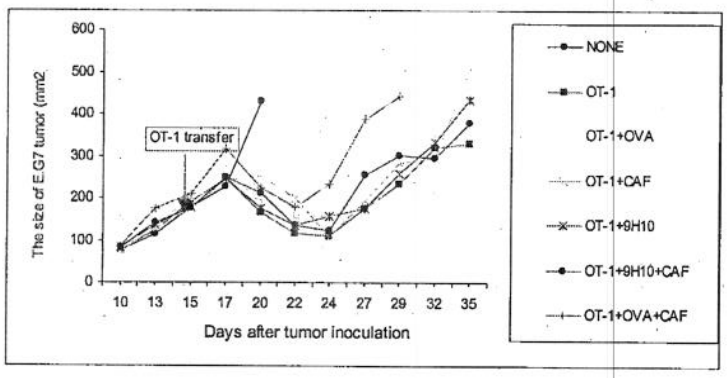


**Figure 3**

Unfortunately, as before, we observed little evidence that systemic purigenic receptor blockade was capable of promoting antigen-specific antitumoral responses against OVA expressing B16 melanoma cells even following adoptive transfer of CD8+ T cells specifically recognizing OVA antigen. As such, we conclude that although systemic purigenic blockade may provide some advantages to promote antitumoral T cell responses, these advantanges do not culminate in a robust response resulting in the regression of highly established tumors (at least against murine TC1 prostate cancer and B16 melanoma). Thus, much more compelling evidence will need to be provided in order to translate systemic purigenic blockade into the clinical arena as an adjuctive treatment to support antitumoral immunotherapy.

## KEY RESEARCH ACCOMPLISHMENTS.

- Original Aim 1. **Completed**
- Revised Aim 2. Elucidate mechanism(s) whereby adenosine inhibits costimulatory T cell activation and the mechanism(s) whereby caffeine-mediated purigenic receptor blockade promotes costimulatory T cell activation. **Completed.**
- Revised Aim 3. Test whether in vivo caffeine-mediated blockade of T cell purigenic receptor plus immunotherapy can act synergistically to improve prostate cancer treatment. **Completed.**

## REPORTABLE OUTCOMES TO DATE.

1. Roden, M. Moser and E.D. Kwon. "Androgen withdrawal increases lymphocyte levels and enhances susceptibility of T cells to costimulatory activation to boost antigen-specific immunity". In preparation.

## CONCLUSIONS.

In summary, our initial studies indicated that in vitro blockade of T cell purigenic receptors A2a and A2b using caffeine promotes T cell proliferation and, thereby, might facilitate T cell-mediated immunity against established tumors that tend to produce high levels of adenosine due to hypoxia and upregulated metabolic activity. Moreover, we have now established a viable method for sustained delivery of caffeine into mice to provide prolonged systemic purigenic receptor blockade. Unfortunately, despite encouraging preliminary data and the attractiveness of the theoretical implications of T cell inhibition by adenosine production by established tumors, we conclude that while systemic purigenic blockade may provide some advantages to promote antitumoral T cell responses, these advantanges do not culminate in a robust response resulting in the regression of highly established tumors (at least against murine TC1 prostate cancer and B16 melanoma). Thus, much more compelling evidence will need to be provided in order to translate systemic purigenic blockade into the clinical arena as an adjuctive treatment to support antitumoral immunotherapy.

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